

=> d his

(FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002)

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L1 QUE RNAS? AND (DNA? OR PLASMI?)

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L6 87836 S RNAS? (S) (CLON? OR EXPRESS?) (S) (BACTERI? OR COLI?)
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=> index bioscience medicine

FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED

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=> s rnas? (s) (dna? or plasmid?)

1 FILES SEARCHED...

8 FILES SEARCHED...

15 FILES SEARCHED...

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

FIELD CODE - 'AND' OPERATOR ASSUMED 'RNAS? (S) '

L2 290661 RNAS? (S) (DNA? OR PLASMI?)

=> s l2 (s) (cell? (a) lys?)

2 FILES SEARCHED...

6 FILES SEARCHED...

9 FILES SEARCHED...

11 FILES SEARCHED...

13 FILES SEARCHED...

17 FILES SEARCHED...

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

FIELD CODE - 'AND' OPERATOR ASSUMED 'L19 (S) '

L3 583 L2 (S) (CELL? (A) LYS?)

=> dup rem l3

DUPLICATE IS NOT AVAILABLE IN 'GENBANK, DGENE, FEDRIP'.

ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE

PROCESSING COMPLETED FOR L3

L4 359 DUP REM L3 (224 DUPLICATES REMOVED)

=> focus l4

FOCUS NOT AVAILABLE IN 'GENBANK'.

PROCESSING COMPLETED FOR L4

ANSWERS FROM NON FOCUS FILES PUT AT END OF ANSWER SET.

L5 359 FOCUS L4 1-

=> d ti l5 1-100

L5 ANSWER 1 OF 359 CAPLUS COPYRIGHT 2002 ACS

TI The use of single-stranded DNA and RNase H to promote
quantitative 'hybrid arrest of translation' of mRNA/DNA hybrids
in reticulocyte lysate cell-free translations

L5 ANSWER 2 OF 359 CAPLUS COPYRIGHT 2002 ACS

TI Specific proteins of microorganisms as regulators of enzyme activity

L5 ANSWER 3 OF 359 USPATFULL

TI Process and a device for the isolation of cell components such as
nucleic acids from natural sources

L5 ANSWER 4 OF 359 USPATFULL

TI Process and a device for the isolation of cell components such as
nucleic acids from natural sources

L5 ANSWER 5 OF 359 CAPLUS COPYRIGHT 2002 ACS

TI Activation of antigen-specific cytotoxic T lymphocyte by dendritic cells

L5 ANSWER 6 OF 359 CAPLUS COPYRIGHT 2002 ACS

TI A rapid microscale technique for isolation of recombinant plasmid DNA
suitable for restriction enzyme analysis

L5 ANSWER 7 OF 359 CAPLUS COPYRIGHT 2002 ACS

TI Autoreactive IgG to intracellular proteins in sera of MS patients

L5 ANSWER 8 OF 359 CAPLUS COPYRIGHT 2002 ACS

TI A rapid method for purifying bacterial deoxyribonucleic acid

TI Preparation of mesophilic microorganisms which contain a D-hydantoinase which is active at elevated temperature

L5 ANSWER 351 OF 359 USPATFULL
 TI Recombinant deoxyribonucleic acid which codes for plasminogen activator and method of making plasminogen activator protein therefrom

L5 ANSWER 352 OF 359 USPATFULL
 TI Production of aryl acylamidases

L5 ANSWER 353 OF 359 USPATFULL
 TI Recombinant deoxyribonucleic acid which codes for plasminogen activator

L5 ANSWER 354 OF 359 FEDRIP COPYRIGHT 2002 NTIS
 TI PURCHASE OF A 600 MHZ NMR SPECTROMETER

L5 ANSWER 355 OF 359 GENBANK.RTM. COPYRIGHT 2002
 TITLE (TI): Complete genome sequence of Clostridium perfringens, an anaerobic flesh-eater
 TITLE (TI): Direct Submission

L5 ANSWER 356 OF 359 GENBANK.RTM. COPYRIGHT 2002
 TITLE (TI): Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18
 TITLE (TI): Direct Submission

L5 ANSWER 357 OF 359 GENBANK.RTM. COPYRIGHT 2002
 TITLE (TI): Genome sequence of Yersinia pestis, the causative agent of plague
 TITLE (TI): Direct Submission

L5 ANSWER 358 OF 359 GENBANK.RTM. COPYRIGHT 2002
 TITLE (TI): Complete DNA sequence of a serogroup A strain of Neisseria meningitidis Z2491
 TITLE (TI): Direct Submission

L5 ANSWER 359 OF 359 GENBANK.RTM. COPYRIGHT 2002
 TITLE (TI): Complete DNA sequence of a serogroup A strain of Neisseria meningitidis Z2491
 TITLE (TI): Direct Submission

=> d ti 15 4 6 27 33 36 37 41 47 48 49 58 65 93 116 139 141 237

L5 ANSWER 4 OF 359 USPATFULL
 TI Process and a device for the isolation of cell components such as nucleic acids from natural sources

L5 ANSWER 6 OF 359 CAPLUS COPYRIGHT 2002 ACS
 TI A rapid microscale technique for isolation of recombinant plasmid DNA suitable for restriction enzyme analysis

L5 ANSWER 27 OF 359 MEDLINE
 TI Rapid purification of double-stranded DNA by triple-helix-mediated affinity capture.

L5 ANSWER 33 OF 359 DGENE (C) 2002 THOMSON DERWENT
 TI Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -

L5 ANSWER 36 OF 359 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI CO ISOLATION OF IN-VIVO PHOSPHORUS-32 LABELED SPECIFIC TRANSCRIPTS AND DNA WITHOUT PHENOL EXTRACTION OR NUCLEASE DIGESTION.

L5 ANSWER 37 OF 359 CAPLUS COPYRIGHT 2002 ACS
 TI Effect of ribonuclease on the association of deoxyribonucleic acid with the membrane in Escherichia coli

L5 ANSWER 41 OF 359 LIFESCI COPYRIGHT 2002 CSA
 TI A rapid method for the analysis of plasmid content and copy number in various streptomycetes grown on agar plates.

L5 ANSWER 47 OF 359 MEDLINE
 TI Purification of essentially RNA free plasmid DNA using a modified Escherichia coli host strain expressing ribonuclease A.

L5 ANSWER 48 OF 359 WPIDS (C) 2002 THOMSON DERWENT
 TI Recombinant prodn. of reverse transcriptase in RNase-deficient cells - and subsequent purificn. by cation-exchange chromatography to produce enzyme with low levels of contaminants.

L5 ANSWER 49 OF 359 CAPLUS COPYRIGHT 2002 ACS
 TI Rapid isolation and sequencing of double-stranded plasmid DNA

L5 ANSWER 58 OF 359 DGENE (C) 2002 THOMSON DERWENT
 TI Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -

L5 ANSWER 65 OF 359 USPATFULL
 TI Method for large scale plasmid purification

L5 ANSWER 93 OF 359 USPATFULL
 TI Method for purifying nucleic acids from heterogenous mixtures

L5 ANSWER 116 OF 359 WPIDS (C) 2002 THOMSON DERWENT
 TI Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates.

L5 ANSWER 139 OF 359 USPATFULL
 TI Process for the preparation of endotoxin-free or endotoxin-depleted nucleic acids and/or oligonucleotides for gene therapy

L5 ANSWER 141 OF 359 USPATFULL
 TI Process for the separation and purification of nucleic acids from biological sources

L5 ANSWER 237 OF 359 USPATFULL
 TI Release of intracellular material and the production therefrom of single stranded nucleic acid

=> d ibib abs 15 4 6 27 33 36 37 41 47 48 49 58 65 93 116 139 141 237

L5 ANSWER 4 OF 359 USPATFULL
 ACCESSION NUMBER: 2001:136453 USPATFULL
 TITLE: Process and a device for the isolation of cell components such as nucleic acids from natural sources
 INVENTOR(S): Colpan, Metin, Essen-Kettwig, Germany, Federal Republic of
 PATENT ASSIGNEE(S): Qiagen GmbH, Hilden, Germany, Federal Republic of (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6277648	B1	20010821

APPLICATION INFO.: US 1994-253152 19940602 (8)
RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 1992-EP2774, filed
on 1 Dec 1992

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1991-4139664	19911202
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Kim, John	
LEGAL REPRESENTATIVE:	Jacobson, Price, Holman & Stern, PLLC	
NUMBER OF CLAIMS:	10	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	10 Drawing Figure(s); 7 Drawing Page(s)	
LINE COUNT:	583	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Described is a method of isolating cell components, such as nucleic acids, from natural sources by filtering a sample of the digested natural sources such as cells or cell fragments. The method is characterized in that the sample is passed through a filter, the pore size of which decreases in the direction of flow of the sample through the filter.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 359 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1980:491453 CAPLUS

DOCUMENT NUMBER: 93:91453

TITLE: A rapid microscale technique for isolation of recombinant plasmid DNA suitable for restriction enzyme analysis

AUTHOR(S): Klein, Ronald D.; Selsing, Erik; Wells, Robert D.

CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA

SOURCE: Plasmid (1980), 3(1), 88-91

CODEN: PLSMDX; ISSN: 0147-619X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A simple and rapid microscale technique is described for the isolation of **plasmid DNA** which involves **cell lysis** with PhOH, centrifugation, PhOH extn., EtOH pptn., and **RNase** digestion. The plasmid DNA is of suitable purity and quantity for multiple restriction endonuclease digestions and bacterial transformations. This miniprep procedure is applicable for a variety of types of plasmids ranging in size from 2900 to 18,400 base pairs (bp) and for a no. of Escherichia coli strains. The plasmids are rapidly cleaved by all restriction enzymes (total of 14 tested). Recombinant clones have been screened for insertions .gtoreq.10 bp and .ltoreq.5000 bp. The procedure takes .apprx.3 h and has been routinely used to simultaneously analyze 24 candidate clones. This procedure is reliable and useful for rapid screening of recombinant DNA candidates where anal. by restriction endonuclease digestion is necessary.

L5 ANSWER 27 OF 359 MEDLINE

ACCESSION NUMBER: 93297739 MEDLINE

DOCUMENT NUMBER: 93297739 PubMed ID: 8517544

TITLE: Rapid purification of double-stranded DNA by triple-helix-mediated affinity capture.

AUTHOR: Ji H; Smith L M

CORPORATE SOURCE: Department of Chemistry, University of Wisconsin-Madison 53706.

SOURCE: ANALYTICAL CHEMISTRY, (1993 May 15) 65 (10) 1323-8.

Journal code: 4NR; 0370536. ISSN: 0003-2700.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930806
Last Updated on STN: 19980206
Entered Medline: 19930716

AB A simple and rapid method for the preparation of highly pure **plasmid DNA** has been developed. The **DNA** is directly captured from bacterial **cell lysates** by formation of a triple-helical structure between the **plasmid dsDNA** and a 20-base biotinylated oligonucleotide attached to streptavidin-coated magnetic beads and then eluted from the beads in pH 9 buffer solution. No phenol extraction, ethanol precipitation, **RNase** digestion, or CsCl gradient centrifugation is required. A general purpose cloning vector, pHJ19, was constructed for this application from pUC19 **DNA** by insertion of a 40-base sequence suitable for triple-helix formation. The approach was also found suitable for the purification of lambda bacteriophage **DNA**.

L5 ANSWER 33 OF 359 DGENE (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: AAZ32515 DNA DGENE
TITLE: Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -
INVENTOR: Hanak J A J; Williams S G
PATENT ASSIGNEE: (COBR-N) COBRA THERAPEUTICS LTD.
PATENT INFO: WO 9953018 A2 19991021 91p
APPLICATION INFO: WO 1999-GB1124 19990413
PRIORITY INFO: GB 1998-7922 19980414
US 1998-81726 19980414
GB 1998-17151 19980806

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-620408 [53]

AN AAZ32515 DNA DGENE

AB PCR primers AAZ32514-Z32515 are used to amplify the **RNase I** gene from the cloning vector pCR3.1RNaseI. The PCR product is used in the production of a **plasmid** containing the **RNase I** gene under the control of the lacZ promoter. The **plasmid** is used in the method of the invention which relates to the production of a substantially RNA-free cellular component. The methods involve culturing a cell producing the cellular component and lysing the cells to produce a **cell lysate**, where the lysate contains the cellular component and sufficient **RNase** activity to degrade substantially all of the RNA molecules present in the lysate. The methods can be used for RNA-free production of cellular components, such as **DNA**, proteins, or carbohydrates, which may have commercial or therapeutic value. RNA is a major contaminant of preparations from **cell lysates** that is difficult to remove as it is similar in size and charge to **DNA**. The invention helps to overcome the limitations of previous methods for RNA removal.

L5 ANSWER 36 OF 359 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1982:225851 BIOSIS
DOCUMENT NUMBER: BA73:85835
TITLE: CO ISOLATION OF IN-VIVO PHOSPHORUS-32 LABELED SPECIFIC TRANSCRIPTS AND DNA WITHOUT PHENOL EXTRACTION OR NUCLEASE DIGESTION.
AUTHOR(S): HAYES S; HAYES C; BRAND L
CORPORATE SOURCE: DEPARTMENT OF MICROBIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF SASKATCHEWAN, SASKATOON, SASKATCHEWAN, S7N 0W0, CANADA.
SOURCE: ANAL BIOCHEM, (1981) 116 (2), 480-488.
CODEN: ANBCA2. ISSN: 0003-2697.

FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A method is described for isolation and quantitation of specific intact

transcripts, for which a hybridization probe is available, from 32P-labeled bacteria [*Escherichia coli*] cells. The RNA is extracted in the absence of **RNase** activity by incorporating an inert, physically removable **RNase** inhibitor throughout the spheroplasting, **cell lysis** and pronase digestion steps. [32P]RNA is separated from [32P]DNA, without recourse to phenol extraction or **DNase** treatment, on a Cs2SO4-HCONH2 step gradient in which the precipitated RNA forms a sharp band. Specific transcripts are purified from [32P]RNA by physical separation of the transcript and hybridization probe using gel-exclusion chromatography. The gentleness of this technique enables the co-isolation of **DNA** and can facilitate the analysis of covalently joined RNA-DNA replication intermediates.

L5 ANSWER 37 OF 359 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1975:424840 CAPLUS

DOCUMENT NUMBER: 83:24840

TITLE: Effect of ribonuclease on the association of deoxyribonucleic acid with the membrane in *Escherichia coli*

AUTHOR(S): McIntosh, Mark A.; Earhart, C. F.

CORPORATE SOURCE: Dep. Microbiol., Univ. Texas, Austin, Tex., USA

SOURCE: J. Bacteriol. (1975), 122(2), 592-8

CODEN: JOBAAY

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The Mg2+-Sarkosyl crystals (M band) procedure was used to study the effect of RNase A on the assocn. of *E. coli* (DNA) with membrane. Incubation of gently prepd. cell exts. with RNase resulted in the release of DNA from membrane. This effect appears to result from the activation, by RNase, of endonuclease I and subsequent limited activity of this DNase. In support of this explanation, it was demonstrated (i) that the extent of the **RNase**-induced loss of **DNA** from membrane was directly correlated with the endogenous level of endonuclease I, and (ii) that endonucleolytic activity occurred when gently lysed cell preps. were incubated in the presence of **RNase**.

L5 ANSWER 41 OF 359 LIFESCI COPYRIGHT 2002 CSA

ACCESSION NUMBER: 90:26328 LIFESCI

TITLE: A rapid method for the analysis of plasmid content and copy number in various streptomycetes grown on agar plates.

AUTHOR: Labes, G.; Simon, R.; Wohlleben, W.

CORPORATE SOURCE: Lehrstuhl Genet., Fak. Biol., Univ. Bielefeld, Postfach 8640, D-4800 Bielefeld 1, FRG

SOURCE: NUCLEIC ACIDS RES., (1990) vol. 18, no. 8, p. 2197.

DOCUMENT TYPE: Journal

FILE SEGMENT: J; N

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The techniques available for the identification of **plasmid DNA** in gram-positive bacteria such as mycelium-forming streptomycetes are time consuming. Here, we present a new rapid and reproducible method by which the bacteria to be lysed can directly be taken from agar plates. For the **cell lysis** a part of a single colony (1-2 mg) is suspended nearly homogeneously in 20 μ l TSE solution (50mM Tris, 10mM NaCl, 5mM EDTA pH 8.0) and gently mixed with 20 μ l L-solution consisting of 25% sucrose, 3% Ficoll 400, 1 Unit **RNase A** and 1 mg/ml lysozyme (freshly dissolved) in TB electrophoresis buffer (90mM Tris, 90mM boric acid, 2.5mM EDTA pH 8.2). Immediately 30 μ l of the suspension is filled into the slot of a submerged 0.2% SDS-containing agarose gel. Complete **cell lysis** is achieved by electrophoretic transfer of the negatively charged SDS into the wells for 40 min at 1V/cm. Electrophoresis is continued for 2-4 h at 10V/cm. Before staining with EtBr the gel is rinsed in water to remove SDS.

L5 ANSWER 47 OF 359 MEDLINE

ACCESSION NUMBER: 2001245167 MEDLINE
DOCUMENT NUMBER: 21094320 PubMed ID: 11173096
TITLE: Purification of essentially RNA free plasmid DNA using a modified Escherichia coli host strain expressing ribonuclease A.
AUTHOR: Cooke G D; Cranenburgh R M; Hanak J A; Dunnill P; Thatcher D R; Ward J M
CORPORATE SOURCE: The Advanced Centre For Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK.
SOURCE: JOURNAL OF BIOTECHNOLOGY, (2001 Feb 23) 85 (3) 297-304. Journal code: AL6; 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010510

AB Regulatory agencies have stringent requirements for the large-scale production of biotherapeutics. One of the difficulties associated with the manufacture of **plasmid DNA** for gene therapy is the removal of the host cell-related impurity RNA following **cell lysis**. We have constructed a modified Escherichia coli JM107 **plasmid** host (JMRNaseA), containing a bovine pancreatic ribonuclease (**RNaseA**) expression cassette, integrated into the host chromosome at the dif locus. The expressed **RNaseA** is translocated to the periplasm of the cell, and is released during primary **plasmid** extraction by alkaline lysis. The **RNaseA** protein is stable throughout incubation at high pH (approximately 12-12.5), and subsequently acts to hydrolyse host cell RNA present in the neutralised solution following alkaline lysis. Results with this strain harbouring pUC18, and a 2.4 kb pUC18DeltalacO, show that sufficient levels of ribonuclease (**RNase**) activity are produced to hydrolyse the bulk of the host RNA. This provides a suitable methodology for the removal of RNA, whilst avoiding the addition of exogenous animal sourced **RNase** and its associated regulatory requirements.

L5 ANSWER 48 OF 359 WPIDS (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: 1995-358626 [46] WPIDS
DOC. NO. CPI: C1995-156896
TITLE: Recombinant prodn. of reverse transcriptase in RNase-deficient cells - and subsequent purificn. by cation-exchange chromatography to produce enzyme with low levels of contaminants.
DERWENT CLASS: B04 D16
INVENTOR(S): KACIAN, D L; PUTNAM, J G; RIGGS, M G; PUTNAM, J
PATENT ASSIGNEE(S): (GENP-N) GEN-PROBE INC
COUNTRY COUNT: 18
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9527047	A2	19951012	(199546)*	EN	53
W: AU CA JP KR					
AU 9522371	A	19951023	(199605)		
EP 688870	A1	19951227	(199605)	EN	42
R: AT BE CH DE DK ES FR GB IT LI LU NL SE					
WO 9527047	A3	19951026	(199621)		
JP 09508806	W	19970909	(199746)		74
KR 97702362	A	19970513	(199821)		
AU 696497	B	19980910	(199848)		
US 5935833	A	19990810	(199938)		

US 5998195 A 19991207 (200004)
 KR 262258 B1 20000715 (200131)
 CA 2186018 C 20020212 (200221) EN

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9527047	A2	WO 1995-US4092	19950329
AU 9522371	A	AU 1995-22371	19950329
EP 688870	A1	EP 1995-104959	19950403
WO 9527047	A3	WO 1995-US4092	19950329
JP 09508806	W	JP 1995-525899	19950329
		WO 1995-US4092	19950329
KR 97702362	A	WO 1995-US4092	19950329
		KR 1996-705427	19960930
AU 696497	B	AU 1995-22371	19950329
US 5935833	A Cont of	US 1994-221804	19940401
		US 1997-778217	19970109
US 5998195	A Cont of	US 1994-221804	19940401
	Cont of	US 1995-443781	19950518
		US 1997-821948	19970321
KR 262258	B1	WO 1995-US4092	19950329
		KR 1996-705427	19960930
CA 2186018	C	CA 1995-2186018	19950329
		WO 1995-US4092	19950329

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9522371	A Based on	WO 9527047
JP 09508806	W Based on	WO 9527047
KR 97702362	A Based on	WO 9527047
AU 696497	B Previous Publ.	AU 9522371
	Based on	WO 9527047
CA 2186018	C Based on	WO 9527047

PRIORITY APPLN. INFO: US 1994-221804 19940401; US 1997-778217
 19970109; US 1995-443781 19950518; US
 1997-821948 19970321

AN 1995-358626 [46] WPIDS

AB WO 9527047 A UPAB: 19951122

A method for producing a polypeptide (A) with RNA-directed and DNA directed DNA polymerase activities, comprises: (a) constructing a **plasmid** contg. a gene encoding (A), at least one selectable marker gene, a promoter sequence, and a replicon capable of autonomously replicating the **plasmid** within a suitable host cell; (b) inserting the **plasmid** into a suitable host cell, deficient in the expression of RNase activity (pref. E.coli strain 1200) ; (c) growing the host cells contg. the **plasmid** in a liq. culture under conditions capable of promoting cell division and polypeptide gene expression; (d) lysing the host cells; and (e) purifying (A) from the **cell lysate**.

USE - (A) is a reverse transcriptase, useful for commercial applications in amplification reactions.

ADVANTAGE - Recombinant prodn. of the reverse transcriptase in RNase-deficient host strains and subsequent purificn. results in an enzyme contg. low levels of contaminants that otherwise interfere with transcription-based amplification reactions.

Dwg.0/10

L5 ANSWER 49 OF 359 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:509959 CAPLUS

DOCUMENT NUMBER: 119:109959

TITLE: Rapid isolation and sequencing of double-stranded plasmid DNA
AUTHOR(S): Akella, Rama; Porter, Rebecca
CORPORATE SOURCE: Guthrie Found. Med. Res., Guthrie Clin./Med. Cent., Sayre, PA, 18840, USA
SOURCE: BioTechniques (1993), 14(5), 726, 728, 730
CODEN: BTNQDO; ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This report describes a rapid method to sequence unpurified supercoiled plasmid DNA prep'd. by a modification of the rapid boiling method described by Holms, D. S. and Quigley, M. (1981). This method eliminates the use of lysozyme for bacterial **cell lysis** and does not require phenol and chloroform extns. or **RNase** digestion prior to the use of the template in **DNA** sequencing reactions. DNA thus made is suitable for restriction anal., if necessary, to verify the presence of an insert of interest in the clone selected before sequencing. Further, co-pptn. of the primer with the denatured template eliminates the extra time required to incubate the template-primer mixt. to anneal the 2 together. The DNA prep'd. by this method also permits sequencing gels run with 35S-dATP to be dried immediately after the run without any further manipulations and exposed to the X-ray film without sacrificing any clarity or intensity of the resolved bands. Beginning with overnight cultures, the entire process of verification for presence of an insert in the selected clone up to loading the sequencing gel can be performed in under 4 h using the procedure described here.

L5 ANSWER 58 OF 359 DGENE (C) 2002 THOMSON DERWENT

ACCESSION NUMBER: AAZ32510 DNA DGENE
TITLE: Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -
INVENTOR: Hanak J A J; Williams S G
PATENT ASSIGNEE: (COBR-N) COBRA THERAPEUTICS LTD.
PATENT INFO: WO 9953018 A2 19991021 91p
APPLICATION INFO: WO 1999-GB1124 19990413
PRIORITY INFO: GB 1998-7922 19980414
US 1998-81726 19980414
GB 1998-17151 19980806
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-620408 [53]

AN AAZ32510 DNA DGENE

AB PCR primers AAZ32510-Z32511 are used to amplify the **RNase A** gene from the expression vector pQR163. The PCR product is used in the production of the pN1RNase A construct. The construct is used in the method of the invention which relates to the production of a substantially RNA-free cellular component. The methods involve culturing a cell producing the cellular component and lysing the cells to produce a **cell lysate**, where the lysate contains the cellular component and sufficient **RNase** activity to degrade substantially all of the RNA molecules present in the lysate. The methods can be used for RNA-free production of cellular components, such as **DNA**, proteins, or carbohydrates, which may have commercial or therapeutic value. RNA is a major contaminant of preparations from **cell lysates** that is difficult to remove as it is similar in size and charge to **DNA**. The invention helps to overcome the limitations of previous methods for RNA removal.

L5 ANSWER 65 OF 359 USPATFULL

ACCESSION NUMBER: 2002:3854 USPATFULL
TITLE: Method for large scale plasmid purification
INVENTOR(S): Lee, Ann L., Lansdale, PA, UNITED STATES
Sagar, Sangeetha, Lansdale, PA, UNITED STATES
PATENT ASSIGNEE(S): Merck & Co., Inc., Rahway, NJ, UNITED STATES, 07065 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002001829	A1	20020103
APPLICATION INFO.:	US 2001-799906	A1	20010306 (9)
RELATED APPLN. INFO.:	Division of Ser. No. US 1997-952428, filed on 7 Nov 1997, GRANTED, Pat. No. US 6197553 A 371 of International Ser. No. WO 1996-US7083, filed on 15 May 1996, UNKNOWN Continuation-in-part of Ser. No. US 1995-446118, filed on 19 May 1995, ABANDONED Continuation-in-part of Ser. No. US 1994-275571, filed on 15 Jul 1994, ABANDONED		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	MERCK AND CO INC, P O BOX 2000, RAHWAY, NJ, 070650907		
NUMBER OF CLAIMS:	11		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	9 Drawing Page(s)		
LINE COUNT:	597		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A process is disclosed for the large scale isolation and purification of plasmid DNA from large scale microbial fermentations. All three forms of plasmid DNA; supercoil (form I), nicked or relaxed circle (form II), and linearized (form III), are individually isolatable using the disclosed process. Highly purified DNA suitable for inclusion in a pharmaceutical composition is provided by the disclosed process.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 93 OF 359 USPATFULL
 ACCESSION NUMBER: 1998:86053 USPATFULL
 TITLE: Method for purifying nucleic acids from heterogenous mixtures
 INVENTOR(S): Gonzalez, Diana, Placentia, CA, United States
 PATENT ASSIGNEE(S): Beckman Instruments, Inc., Fullerton, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5783686		19980721
APPLICATION INFO.:	US 1995-529148		19950915 (8)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Wilson, James O.		
LEGAL REPRESENTATIVE:	May, William H., Harder, P. R. Fulbright & Jaworski		
NUMBER OF CLAIMS:	16		
EXEMPLARY CLAIM:	1		
LINE COUNT:	641		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Discloses DNA isolation and purification methods which involve novel washing steps. The disclosed methods provide a means for isolating and purifying DNA from a homogeneous mixture of DNA of other cellular contaminants by treating silica with the homogeneous mixture containing DNA in the presence of a chaotropic salt solution and then washing and separating the washed and treated silica in successive wash steps with aqueous alcohol wash solutions. A first wash step involves washing the treated silica with a first wash solution of at least 95 wt % alcohol in water. A second wash step similarly involves washing the treated and washed silica with second wash solution of less than 95 wt % alcohol in water.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 116 OF 359 WPIDS (C) 2002 THOMSON DERWENT
 ACCESSION NUMBER: 1999-620408 [53] WPIDS

DOC. NO. CPI: C1999-181161
 TITLE: Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates.
 DERWENT CLASS: B04 D16
 INVENTOR(S): HANAK, J A J; WILLIAMS, S G
 PATENT ASSIGNEE(S): (COBR-N) COBRA THERAPEUTICS LTD
 COUNTRY COUNT: 87
 PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9953018	A2	19991021	(199953)*	EN	91
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9934348	A	19991101	(200013)		
EP 1080179	A2	20010307	(200114)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9953018	A2	WO 1999-GB1124	19990413
AU 9934348	A	AU 1999-34348	19990413
EP 1080179	A2	EP 1999-915927	19990413
		WO 1999-GB1124	19990413

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9934348	A Based on	WO 9953018
EP 1080179	A2 Based on	WO 9953018

PRIORITY APPLN. INFO: GB 1998-17151 19980806; GB 1998-7922
 19980414; US 1998-81726P 19980414

AN 1999-620408 [53] WPIDS

AB WO 9953018 A UPAB: 19991215

NOVELTY - Novel methods are described for producing a substantially RNA-free cellular component.

DETAILED DESCRIPTION - A method of preparing a substantially RNA-free cellular component is new, and comprises culturing cell producing the cellular component (A) in a medium, and lysing the cells to produce a cell lysate, wherein the cell lysate contains (A) and sufficient RNase activity to degrade substantially all of the RNA molecules present in the lysate.

INDEPENDENT CLAIMS are also included for the following:

- (1) a method of preparing a substantially RNA-free (A), comprising culturing and lysing cells producing (A) and cells producing RNase in an amount sufficient to degrade substantially all of the RNA present in the preparation;
- (2) a host cell that produces a recombinant DNA, a recombinant protein, or a recombinant carbohydrate, and also produces an RNase in a regulated manner;
- (3) a composition comprising the host cell of (2) or
- (4) a pharmaceutical composition comprising (A) that is substantially RNA-free, in a pharmaceutically acceptable carrier.

USE - The methods can be used for RNA-free production of cellular components, such as DNA, proteins, or carbohydrates, which may have commercial or therapeutic value.

ADVANTAGE - RNA is a major contaminant of preparations from cell lysates that is difficult to remove as it is

similar in size and charge to DNA. Prior art methods used exogenously produced RNase to remove this RNA. However, there are limitations to using exogenously produced RNase, in that it is difficult to purify in large amounts and so is expensive to produce. The invention provides methods for RNA-free purification of cellular components that overcome these limitations.
Dwg.0/19

L5 ANSWER 139 OF 359 USPATFULL
ACCESSION NUMBER: 2002:55160 USPATFULL
TITLE: Process for the preparation of endotoxin-free or endotoxin-depleted nucleic acids and/or oligonucleotides for gene therapy
INVENTOR(S): Colpan, Metin, Essen, GERMANY, FEDERAL REPUBLIC OF
Schorr, Joachim, Dusseldorf, GERMANY, FEDERAL REPUBLIC OF
Moritz, Peter, Kerpen, GERMANY, FEDERAL REPUBLIC OF
PATENT ASSIGNEE(S): QIAGEN GMBH

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002032324	A1	20020314
APPLICATION INFO.:	US 2001-962459	A1	20010926 (9)
RELATED APPLN. INFO.:	Division of Ser. No. US 1999-253702, filed on 22 Feb 1999, GRANTED, Pat. No. US 6297371 Division of Ser. No. US 1996-687529, filed on 18 Oct 1996, GRANTED, Pat. No. US 5990301 A 371 of International Ser. No. WO 1995-EP389, filed on 3 Feb 1995, UNKNOWN		

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1994-4403692	19940207
	DE 1994-4422291	19940625
	DE 1994-4431125	19940901
	DE 1994-4432654	19940914
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	JACOBSON HOLMAN PLLC, 400 SEVENTH STREET N.W., SUITE 600, WASHINGTON, DC, 20004	
NUMBER OF CLAIMS:	17	
EXEMPLARY CLAIM:	1	
LINE COUNT:	702	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A process for the isolation and purification of nucleic acids and/or oligonucleotides for use in gene therapy wherein said nucleic acids and/or oligonucleotides are isolated or purified from an essentially biological source, characterized in that

said essentially biological sources are lysed, the fractions obtained are optionally freed or depleted from the remainder of said biological sources by per se known mechanical methods, such as centrifugation, filtration;

the fractions thus treated are subsequently treated with affinity chromatographic material or with inorganic chromatographic material for the removal of endotoxins; followed by

isolation of said nucleic acids and/or oligonucleotides on an anion exchanger which is designed such that DNA begins to desorb from the anion exchanger only at an ionic strength corresponding to a sodium chloride solution of a concentration higher by at least 100 mM than one corresponding to the ionic strength at which RNA begins to desorb from the anion exchanger material.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 141 OF 359 USPATFULL
 ACCESSION NUMBER: 1999:151401 USPATFULL
 TITLE: Process for the separation and purification of nucleic acids from biological sources
 INVENTOR(S): Colpan, Metin, Essen, Germany, Federal Republic of
 Schorr, Joachim, Dusseldorf, Germany, Federal Republic of
 Moritz, Peter, Kerpen, Germany, Federal Republic of
 PATENT ASSIGNEE(S): Qiagen GmbH, Hilden, Germany, Federal Republic of
 (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5990301		19991123
	WO 9521177		19950810
APPLICATION INFO.:	US 1996-687529		19961018 (8)
	WO 1995-EP389		19950203
			19961018 PCT 371 date
			19961018 PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1994-4403692	19940207
	DE 1994-4422291	19940625
	DE 1994-4431125	19940914
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Wilson, James O.	
LEGAL REPRESENTATIVE:	Jacobson, Price, Holman & Stern, PLLC	
NUMBER OF CLAIMS:	17	
EXEMPLARY CLAIM:	1	
LINE COUNT:	681	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

AB A process for the isolation and purification of nucleic acids and/or oligonucleotides for use in gene therapy wherein nucleic acids and/or oligonucleotides are isolated or purified from an essentially biological source, characterized in that

essentially biological sources are lysed, the fractions obtained are optionally freed or depleted from the remainder of biological sources by per se known mechanical methods, such as centrifugation, filtration;

the fractions thus treated are subsequently treated with affinity chromatographic material or with inorganic chromatographic material for the removal of endotoxins; followed by

isolation of nucleic acids and/or oligonucleotides on an anion exchanger which is designed such that DNA begins to desorb from the anion exchanger only at an ionic strength corresponding to a sodium chloride solution of a concentration higher by at least 100 mM than one corresponding to the ionic strength at which RNA begins to desorb from the anion exchanger material.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 237 OF 359 USPATFULL
 ACCESSION NUMBER: 2002:1067 USPATFULL
 TITLE: Release of intracellular material and the production therefrom of single stranded nucleic acid
 INVENTOR(S): Martin, Sophie E.V., Cambridge, UNITED KINGDOM
 Bergmann, Karin, Cambridge, UNITED KINGDOM
 Pollard-Knight, Denise V., London, UNITED KINGDOM
 PATENT ASSIGNEE(S): Scientific Generics Limited, Cambridge, UNITED KINGDOM
 (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6335161	B1	20020101
APPLICATION INFO.:	US 1998-30028		19980225 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. WO 1995-GB2024, filed on 25 Aug 1995		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Horlick, Kenneth R.		
ASSISTANT EXAMINER:	Tung, Joyce		
LEGAL REPRESENTATIVE:	Pillsbury Winthrop LLP		
NUMBER OF CLAIMS:	6		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	5 Drawing Figure(s); 4 Drawing Page(s)		
LINE COUNT:	586		
CAS INDEXING IS AVAILABLE FOR THIS PATENT.			
AB	Intracellular material is released from bacterial, yeast, plant, animal, insect or human cells by the application of a low voltage such as 1 to 10 V to a suspension containing the cells. The conditions may be selected such that DNA released from the cells is electrochemically denatured so as to be available for use in an amplification procedure.		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:24:09 ON 06 JUN 2002

SEA RNAS? AND (DNA? OR PLASMI?)

8	FILE ADISALERTS
9	FILE ADISINSIGHT
844	FILE AGRICOLA
19	FILE ANABSTR
231	FILE AQUASCI
108	FILE BIOBUSINESS
43	FILE BIOCOMMERCE
10723	FILE BIOSIS
463	FILE BIOTECHABS
463	FILE BIOTECHDS
6946	FILE BIOTECHNO
1779	FILE CABA
4020	FILE CANCERLIT
14914	FILE CAPLUS
103	FILE CEABA-VTB
32	FILE CEN
22	FILE CIN
19	FILE CONFSCI
2	FILE CROPB
25	FILE CROPU
4	FILE DDFB
123	FILE DDFU
39403	FILE DGENE
4	FILE DRUGB
7	FILE DRUGNL
260	FILE DRUGU
6	FILE DRUGUPDATES
44	FILE EMBAL

8355 FILE EMBASE
 3511 FILE ESBIODBASE
 548 FILE FEDRIP
 9 FILE FROSTI
 68 FILE FSTA
 195265 FILE GENBANK
 463 FILE IFIPAT
 524 FILE JICST-EPLUS
 3 FILE KOSMET
 4474 FILE LIFESCI
 11798 FILE MEDLINE
 33 FILE NIOSHTIC
 99 FILE NTIS
 38 FILE OCEAN
 1644 FILE PASCAL
 16 FILE PHAR
 1 FILE PHIC
 18 FILE PHIN
 265 FILE PROMT
 8607 FILE SCISEARCH
 3279 FILE TOXCENTER
 14388 FILE USPATFULL
 52 FILE USPAT2
 590 FILE WPIDS
 590 FILE WPINDEX
 8 FILE NAPRALERT
 429 FILE NLDB

L1 QUE RNAS? AND (DNA? OR PLASMI?)

FILE 'GENBANK, DGENE, CAPLUS, USPATFULL, MEDLINE, BIOSIS, SCISEARCH,
 EMBASE, BIOTECHNO, LIFESCI, CANCERLIT, ESBIODBASE, TOXCENTER, CABA,
 PASCAL, AGRICOLA, WPIDS, FEDRIP, JICST-EPLUS' ENTERED AT 16:26:32 ON 06
 JUN 2002

L2 290661 S RNAS? (S) (DNA? OR PLASMI?)
 L3 583 S L2 (S) (CELL? (A) LYS?)
 L4 359 DUP REM L3 (224 DUPLICATES REMOVED)
 L5 359 FOCUS L4 1-

=> s rnas? (s) (clon? or express?) (s) (bacteri? or coli?)

1 FILES SEARCHED...
 3 FILES SEARCHED...
 8 FILES SEARCHED...
 11 FILES SEARCHED...
 15 FILES SEARCHED...

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
 FIELD CODE - 'AND' OPERATOR ASSUMED 'RNAS? (S) '
 PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
 FIELD CODE - 'AND' OPERATOR ASSUMED 'EXPRESS?) (S) '

L6 87836 RNAS? (S) (CLON? OR EXPRESS?) (S) (BACTERI? OR COLI?)

=> s l6 (s) (gen?)

1 FILES SEARCHED...
 2 FILES SEARCHED...
 5 FILES SEARCHED...
 7 FILES SEARCHED...
 9 FILES SEARCHED...
 11 FILES SEARCHED...
 12 FILES SEARCHED...
 15 FILES SEARCHED...
 17 FILES SEARCHED...

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
 FIELD CODE - 'AND' OPERATOR ASSUMED 'L61 (S) '

L7 59604 L6 (S) (GEN?)

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SEARCH ENDED BY USER

=> s l7 (s) secret? (s) periplasm?

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

FIELD CODE - 'AND' OPERATOR ASSUMED 'L81 (S) SECRET?'

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH

FIELD CODE - 'AND' OPERATOR ASSUMED 'SECRET? (S) PERIPLASM'

18 FILES SEARCHED...

L8 23 L7 (S) SECRET? (S) PERIPLASM?

=> dup rem l8

DUPLICATE IS NOT AVAILABLE IN 'GENBANK, DGENE, FEDRIP'.

ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE

PROCESSING COMPLETED FOR L8

L9 11 DUP REM L8 (12 DUPLICATES REMOVED)

=> d ti l9 1-11

L9 ANSWER 1 OF 11 USPATFULL

TI Ligand for vascular endothelial growth factor receptor

L9 ANSWER 2 OF 11 USPATFULL

TI ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES

L9 ANSWER 3 OF 11 USPATFULL

TI Production and use of recombinant protein multimers with increased biological activity

L9 ANSWER 4 OF 11 USPATFULL

TI DNA encoding erythropoietin multimers having modified 5' and 3' sequences and its use to prepare EPO therapeutics

L9 ANSWER 5 OF 11 USPATFULL

TI Expression of functional antibody fragments

L9 ANSWER 6 OF 11 MEDLINE DUPLICATE 1

TI Relatedness of a periplasmic, broad-specificity RNase from *Aeromonas hydrophila* to RNase I of *Escherichia coli* and to a family of eukaryotic RNases.

L9 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2

TI Secretion of recombinant ribonuclease T1 into the periplasmic space of *Escherichia coli* with the aid of the signal peptide of alkaline phosphatase

L9 ANSWER 8 OF 11 MEDLINE DUPLICATE 3

TI Expression of *Bacillus amyloliquefaciens* extracellular ribonuclease (barnase) in *Escherichia coli* following an inactivating mutation.

L9 ANSWER 9 OF 11 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491

TITLE (TI): Direct Submission

L9 ANSWER 10 OF 11 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491

TITLE (TI): Direct Submission

L9 ANSWER 11 OF 11 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): The genome sequence of the food-borne pathogen
Campylobacter jejuni reveals hypervariable sequences
TITLE (TI): Direct Submission

=> d 19 6 7 8 ibib abs

L9 ANSWER 6 OF 11 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 93285983 MEDLINE
DOCUMENT NUMBER: 93285983 PubMed ID: 7685334
TITLE: Relatedness of a periplasmic, broad-specificity RNase from
Aeromonas hydrophila to RNase I of Escherichia coli and to
a family of eukaryotic RNases.
AUTHOR: Favre D; Ngai P K; Timmis K N
CORPORATE SOURCE: Departement de Biochimie Medicale, Centre Medical
Universitaire, Geneva, Switzerland.
SOURCE: JOURNAL OF BACTERIOLOGY, (1993 Jun) 175 (12) 3710-22.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
JOURNAL; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X67054
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19970203
Entered Medline: 19930713

AB The isolation, sequencing, and characterization of a **periplasmic RNase gene** from *Aeromonas hydrophila* AH1133 is described. Following subcloning of the **gene** on a 2.7-kb PstI fragment, its direction of transcription and approximate location were determined. Analysis of the nucleotide sequence reveals that the **gene** is 645 bp long, coding for 215 amino acid residues with a total molecular weight of 24,215. A typical leader sequence is present at the beginning of the corresponding protein. Computer analysis revealed strong local similarities to *Escherichia coli* **RNase I** and to the active site of a family of eukaryotic **RNases**. **Expression** studies indicate that the **RNase** natural promoter functions poorly in *E. coli*. In this organism, the enzyme is mainly localized in the cytoplasm and **periplasm**, although high levels of **expression** lead to significant release into the extracellular medium. Functional and physical characterizations further indicate that the **periplasmic** and cytoplasmic enzymes of *A. hydrophila* are likely to be the counterparts of *E. coli* **RNase I** and its cytoplasmic form **RNase I***: as for the *E. coli* enzymes, the *A. hydrophila* **RNase** forms have similar sizes and show broad specificity, and the **periplasmic** form is more active towards natural polymer RNA than its cytoplasmic counterpart. Both forms are relatively thermosensitive and are reversibly inactivated by up to 0.6% sodium dodecyl sulfate. Southern hybridization revealed homology to *E. coli* K-12 and *Shigella* sp. **genomic DNA**, a finding which correlates with the presence of **secreted RNases** in these organisms. In contrast, species of phylogenetically closer **genera**, such as *Vibrio* and *Plesiomonas*, did not hybridize to the *A. hydrophila* **RNase gene**.

L9 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 1990:606105 CAPLUS
DOCUMENT NUMBER: 113:206105
TITLE: Secretion of recombinant ribonuclease T1 into the
periplasmic space of *Escherichia coli* with the aid of
the signal peptide of alkaline phosphatase
AUTHOR(S): Fujimura, Takao; Tanaka, Toshiki; Ohara, Kanako;
Morioka, Hiroshi; Uesugi, Seiichi; Ikehara, Morio;
Nishikawa, Satoshi

CORPORATE SOURCE: Fac. Pharm. Sci., Osaka Univ., Suita, 565, Japan
SOURCE: FEBS Lett. (1990), 265(1-2), 71-4
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The RNase T1 gene of *Aspergillus oryzae* was ligated to a synthetic gene for the signal peptide of *E. coli* alk. phosphatase. When this fusion gene was **expressed** in *E. coli* under the control of the trp promoter, active RNase T1 having the correct N-terminal sequence was **secreted** into the **periplasmic** space, indicating that the heterologous signal peptide had been cleaved off correctly. The enzyme could be readily purified from the periplasmic fraction with a yield of 1.8 mg from 1 L culture. Adopting the same strategy, it was possible to produce a labile mutant of RNase T1 (Glu-58 .fwdarw. Ala mutant) in *E. coli*, the yield of the purified mutant enzyme being 2.0 mg from 1 L culture.

L9 ANSWER 8 OF 11 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 87248102 MEDLINE
DOCUMENT NUMBER: 87248102 PubMed ID: 3297926
TITLE: Expression of *Bacillus amyloliquefaciens* extracellular ribonuclease (barnase) in *Escherichia coli* following an inactivating mutation.
AUTHOR: Paddon C J; Hartley R W
SOURCE: GENE, (1987) 53 (1) 11-9.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198708
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19870805

AB An inactivated gene for *Bacillus amyloliquefaciens* extracellular ribonuclease (barnase) has previously been **cloned** and sequenced following transposon mutagenesis. The intact gene could not be assembled in *Escherichia coli* and is presumed to be lethal. Therefore, we introduced specific mutations into the barnase gene to prevent its lethal effect. A Gln-73 mutant gene was stable in *E. coli* but only produced low amounts of barnase antigen. Mutants containing Asp, Gln or Arg, instead of His-102, at the active site were identified by immunological screening for barnase antigen. None of the mutant proteins with alterations at aa residue 102 possessed RNase activity. The level of barnase (Asp-102) was higher in *E. coli* than in *B. subtilis* but the protein was not processed to the correct size in *E. coli*. To obtain correct processing, the barnase (Asp-102) structural gene was fused to the *E. coli* alkaline phosphatase promoter and signal sequence (phoA). Cells containing this construct **secreted** correctly processed barnase (Asp-102) into the **periplasmic** space and culture supernatant at a level of 20 mg/l. Barnase (Asp-102) was purified and found to have an identical N-terminus and a thermal unfolding curve that was nearly identical to that of active barnase (His-102). The **cloning** and **expression** of barnase in *E. coli* will allow detailed analysis of barnase protein folding by molecular **genetic** approaches.

=> d his

(FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCCommerce, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,

CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:24:09 ON 06 JUN 2002

SEA RNAS? AND (DNA? OR PLASMI?)

8 FILE ADISALERTS
9 FILE ADISINSIGHT
844 FILE AGRICOLA
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14914 FILE CAPLUS
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123 FILE DDFU
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548 FILE FEDRIP
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68 FILE FSTA
195265 FILE GENBANK
463 FILE IFIPAT
524 FILE JICST-EPLUS
3 FILE KOSMET
4474 FILE LIFESCI
11798 FILE MEDLINE
33 FILE NIOSHTIC
99 FILE NTIS
38 FILE OCEAN
1644 FILE PASCAL
16 FILE PHAR
1 FILE PHIC
18 FILE PHIN
265 FILE PROMT
8607 FILE SCISEARCH
3279 FILE TOXCENTER
14388 FILE USPATFULL
52 FILE USPAT2
590 FILE WPIDS
590 FILE WPINDEX
8 FILE NAPRALERT
429 FILE NLDB

L1

QUE RNAS? AND (DNA? OR PLASMI?)

FILE 'GENBANK, DGENE, CAPLUS, USPATFULL, MEDLINE, BIOSIS, SCISEARCH, EMBASE, BIOTECHNO, LIFESCI, CANCERLIT, ESBIODBASE, TOXCENTER, CABA,

PASCAL, AGRICOLA, WPIDS, FEDRIP, JICST-EPLUS' ENTERED AT 16:26:32 ON 06
JUN 2002

L2 290661 S RNAS? (S) (DNA? OR PLASMI?)
L3 583 S L2 (S) (CELL? (A) LYS?)
L4 359 DUP REM L3 (224 DUPLICATES REMOVED)
L5 359 FOCUS L4 1-
L6 87836 S RNAS? (S) (CLON? OR EXPRESS?) (S) (BACTERI? OR COLI?)
L7 59604 S L6 (S) (GEN?)
L8 23 S L7 (S) SECRET? (S) PERIPLASM?
L9 11 DUP REM L8 (12 DUPLICATES REMOVED)

=> log h

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FULL ESTIMATED COST	169.63	171.96
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	ENTRY	SESSION
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